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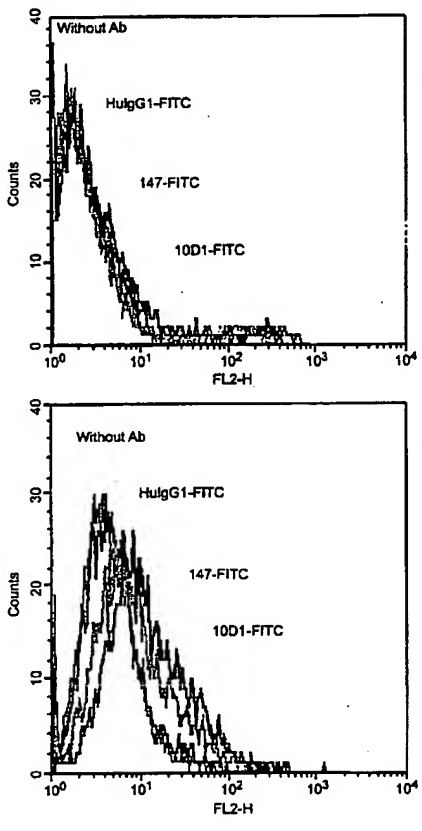

(57) Abstract: The present invention provides novel human sequence antibodies against human CTLA-4 and methods of treating human diseases, infections and other conditions using these antibodies.

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HUMAN CTLA-4 ANTIBODIES AND THEIR USES

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional patent application Serial No. 60/150,452, the disclosure of which is incorporated herein in its entirety.

FIELD OF THE INVENTION

The present invention relates generally to molecular immunology and the treatment of human diseases. In particular, it relates to novel human sequence antibodies against human CTLA-4 and methods of treating human diseases and infections using these antibodies.

BACKGROUND OF THE INVENTION

The vertebrate immune system requires multiple signals to achieve optimal immune activation; *see, e.g.*, Janeway, Cold Spring Harbor Symp. *Quant. Biol.* 54:1-14 (1989); Paul William E., ed. Raven Press, N.Y., *Fundamental Immunology,* 4th edition (1998), particularly chapters 12 and 13, pages 411 to 478. Interactions between T lymphocytes (T cells) and antigen presenting cells (APC) are essential to the immune response. Levels of many cohesive molecules found on T cells and APC's increase during an immune response (Springer *et al..*, *A. Rev. Immunol.* 5:223-252 (1987); Shaw and Shimuzu, *Current Opinion in Immunology*, Eds. Kindt and Long, 1:92-97 (1988)); and Hemler, *Immunology Today* 9:109-113 (1988)). Increased levels of these molecules may help explain why activated APC's are more effective at stimulating antigen-specific T cell proliferation than are resting APC's (Kaiuchi *et al..*, *J. Immunol.* 131:109-114 (1983); Kreiger *et al..*, *J. Immunol.* 135:2937-2945 (1985); McKenzie, *J. Immunol.* 141:2907-2911 (1988); and Hawrylowicz and Unanue, *J. Immunol.* 141:4083-4088 (1988)).

T cell immune response is a complex process that involves cell-cell interactions (Springer *et al..*, *A. Rev. Immunol.* 5:223-252 (1987)), particularly between T and accessory cells such as APC's, and production of soluble immune mediators (cytokines or lymphokines) (Dinarello (1987) *New Engl. Jour. Med* 317:940-945; Sallusto (1997) *J. Exp. Med.* 179:1109-1118). This response is regulated by several T-cell surface receptors, including the T-cell receptor complex (Weiss (1986) *Ann. Rev. Immunol.* 4:593-619) and other "accessory" surface molecules (Allison (1994) *Curr.*

Opin. Immunol. 6:414-419; Springer (1987) *supra*). Many of these accessory molecules are naturally occurring cell surface differentiation (CD) antigens defined by the reactivity of monoclonal antibodies on the surface of cells (McMichael, Ed., *Leukocyte Typing III*, Oxford Univ. Press, Oxford, N.Y. (1987)).

Early studies suggested that B lymphocyte activation requires two signals (Bretscher (1970) *Science* 169:1042-1049) and now it is believed that all lymphocytes require two signals for their optimal activation, an antigen specific or clonal signal, as well as a second, antigen non-specific signal. (Janeway, *supra*). Freeman (1989) *J. Immunol.* 143:2714-2722) isolated and sequenced a cDNA clone encoding a B cell activation antigen recognized by MAb B7 (Freeman (1987) *J. Immunol.* 138:3260). COS cells transfected with this cDNA have been shown to stain by both labeled MAb B7 and MAb BB-1 (Clark (1986) Human Immunol. 16:100-113; Yokochi (1981) *J. Immunol.* 128:823; Freeman *et al..*, (1989) *supra*; Freeman *et al..* (1987), *supra*). In addition, expression of this antigen has been detected on cells of other lineages, such as monocytes (Freeman *et al..*, *supra*).

T helper cell (Th) antigenic response requires signals provided by APC's. The first signal is initiated by interaction of the T cell receptor complex (Weiss, *J. Clin. Invest.* 86:1015 (1990)) with antigen presented in the context of class II major histocompatibility complex (MHC) molecules on the APC (Allen, *Immunol. Today* 8:270 (1987)). This antigen-specific signal is not sufficient to generate a full response, and in the absence of a second signal may actually lead to clonal inactivation or anergy (Schwartz, *Science* 248:1349 (1990)). The requirement for a second "costimulatory" signal provided by the MHC has been demonstrated in a number of experimental systems (Schwartz, *supra*; Weaver and Unanue, *Immunol. Today* 11:49 (1990)). The molecular nature of this second signal is not completely understood, although it is clear in some cases that both soluble molecules such as interleukin (IL)-1 (Weaver and Unanue, *supra*) and membrane receptors involved in intercellular adhesion (Springer, *Nature* 346:425 (1990)) can provide costimulatory signals.

CD28 antigen, a homodimeric glycoprotein of the immunoglobulin superfamily (Aruffo and Seed, *Proc. Natl. Acad. Sci.* 84:8573-8577 (1987)), is an accessory molecule found on most mature human T cells (Damle *et al..*, *J. Immunol.* 131:2296-2300 (1983)). Current evidence suggests that this molecule functions in an alternative T cell activation pathway distinct from that initiated by the T-cell receptor complex (June *et al..*, *Mol. Cell. Biol.* 7:4472-4481 (1987)). Monoclonal antibodies

(MAbs) reactive with CD28 antigen can augment T cell responses initiated by various polyclonal stimuli (reviewed by June *et al.*., *supra*). These stimulatory effects may result from MAb-induced cytokine production (Thompson *et al.*., *Proc. Natl. Acad. Sci* 86:1333-1337 (1989); and Lindsten *et al.*., *Science* 244:339-343 (1989)) as a consequence of increased mRNA stabilization (Lindsten *et al.*. (1989), *supra*). Anti-CD28 mAbs can also have inhibitory effects, *i.e.*, they can block autologous mixed lymphocyte reactions (Damle *et al.*., *Proc. Natl. Acad. Sci.* 78:5096-6001 (1981)) and activation of antigen-specific T cell clones (Lesslauer *et al.*., Eur. *J. Immunol.* 16:1289-1296 (1986)).

Some studies have indicated that CD28 is a counter-receptor for the B cell activation antigen, B7/BB-1 (Linsley *et al.*, *Proc. Natl. Acad. Sci.* USA 87:5031-5035 (1990)). The B7/BB-1 antigen is hereafter referred to as the "B7 antigen". The B7 ligands are also members of the immunoglobulin superfamily but have, in contrast to CD28, two Ig domains in their extracellular region, an N-terminal variable (V)-like domain followed by a constant (C)-like domain.

Delivery of a non-specific costimulatory signal to the T cell requires at least two homologous B7 family members found on APC's, B7-1 (also called B7, B7.1, or CD80) and B7-2 (also called B7.2 or CD86), both of which can deliver costimulatory signals to T cells via CD28. Costimulation through CD28 promotes T cell activation.

Using genetic fusions of the extracellular portions of B7 antigen and CD28 receptor, and Immunoglobulin (Ig) C.gamma.1 (constant region heavy chains), interactions between CD28 and B7 antigen have been characterized (Linsley *et al.*, *J. Exp. Med.* 173:721-730 (1991)). Immobilized B7Ig fusion protein, as well as B7 positive CHO cells, have been shown to costimulate T cell proliferation.

T cell stimulation with B7 positive CHO cells also specifically stimulates increased levels of transcripts for IL-2. Additional studies have shown that anti-CD28 MAb inhibited IL-2 production induced in certain T cell leukemia cell lines by cellular interactions with a B cell leukemia line (Kohno *et al.*., Cell. Immunol. 131-1-10 (1990)).

CD28 has a single extracellular variable region (V)-like domain (Aruffo and Seed, *supra*). A homologous molecule, CTLA-4 has been identified by differential screening of a murine cytolytic-T cell cDNA library (Brunet (1987) *Nature* 328:267-270).

CTLA-4 is a T cell surface molecule that was originally identified by differential screening of a murine cytolytic T cell cDNA library (Brunet *et al.*., *Nature* 328:267-270(1987)). CTLA-4 is also a member of the immunoglobulin (Ig) superfamily; CTLA-4 comprises a single extracellular Ig domain. CTLA-4 transcripts have been

found in T cell populations having cytotoxic activity, suggesting that CTLA-4 might function in the cytolytic response (Brunet *et al.., supra* ; Brunet *et al..*, Immunol. Rev. 103-21-36 (1988)). Researchers have reported the cloning and mapping of a gene for the human counterpart of CTLA-4 (Dariavach *et al.., Eur. J. Immunol.* 18:1901-1905 (1988)) to the same chromosomal region (2q33-34) as CD28 (Lafage-Pochitaloff *et al..*, *Immunogenetics* 31:198-201 (1990)). Sequence comparison between this human CTLA-4 DNA and that encoding CD28 proteins reveals significant homology of sequence, with the greatest degree of homology in the juxtamembrane and cytoplasmic regions (Brunet *et al..*, 1988, *supra*; Dariavach *et al..*, 1988, *supra*).

Some studies have suggested that CTLA-4 has an analogous function as a secondary costimulator (Linsley *et al., J Exp. Med.* 176:1595-1604 (1992); Wu *et al.., J Exp. Med.* 185:1327-1335 (1997) Lindsley, P. *et al..* U.S. Patent Nos. 5,977,318; 5,968,510; 5,885,796; and 5,885,579). However, others have reported that CTLA-4 has an opposing role as a dampener of T cell activation (Krummel (1995) *J. Exp. Med.* 182:459-465); Krummel *et al.., Int'l Immunol.* 8:519-523(1996); Chambers *et al..*, *Immunity*. 7:885-895(1997)). It has been reported that CTLA-4 deficient mice suffer from massive lymphoproliferation (Chambers *et al.., supra*). It has been reported that CTLA-4 blockade augments T cell responses *in vitro* (Walunas *et al.., Immunity*. 1:405-413 (1994)) and *in vivo* (Kearney (1995) *J. Immunol.* 155:1032-1036), exacerbates antitumor immunity (Leach (1996) *Science*. 271:1734-1736), and enhances an induced autoimmune disease (Luhder (1998) *J Exp. Med.* 187:427-432). It has also been reported that CTLA-4 has an alternative or additional impact on the initial character of the T cell immune response (Chambers (1997) *Curr. Opin. Immunol.* 9:396-404; Bluestone (1997) *J. Immunol.* 158:1989-1993; Thompson (1997) *Immunity 7:445-450*). This is consistent with the observation that some autoimmune patients have autoantibodies to CTLA-4. It is possible that CTLA-4 blocking antibodies have a pathogenic role in these patients (Matsui (1999) J. Immunol. 162:4328-4335).

Non-human CTLA-4 antibodies have be used in the various studies discussed above. However, one of the major impediments facing the development of *in vivo* therapeutic and diagnostic applications for antibodies in humans is the intrinsic immunogenicity of non-human immunoglobulins. For example, when immunocompetent human patients are administered therapeutic doses of rodent monoclonal antibodies, the patients produce antibodies against the rodent immunoglobulin sequences; these human anti-mouse antibodies (HAMA) neutralize the therapeutic antibodies and can cause acute

toxicity. These and other deficiencies in the previous antibodies are overcome by the provision of human antibodies to CTLA-4 by the present invention.

SUMMARY OF THE INVENTION

The present invention provides a human sequence antibody that specifically binds to human CTLA-4 and a human sequence antibody that specifically binds to human CTLA-4 which is substantially free of non-immunoglobulin associated human proteins.

In a related aspect, the invention also provides a therapeutically-effective human sequence antibody that specifically binds to human CTLA-4. In some embodiments, the therapeutically-effective human sequence antibody binds to CTLA-4 on the cell surface of normal human T cells. In other embodiments, the T cell subpopulations marked by CD antigens CD4, CD8, CD25, and CD69 remain stable during and subsequent to the administration of the therapeutically-effective human sequence antibody. In other embodiments, the therapeutically-effective human sequence antibody binds CTLA-4 on the cell surface of normal human T cells. In other embodiments, the human sequence antibody well-tolerated in a patient. In a related embodiment,

Also provided is a composition of polyclonal antibodies comprising a plurality of human sequence antibodies that specifically bind to human CTLA-4. The composition of polyclonal antibodies can comprise at least about 2, 5, 10, 50, 100, 500 or 1000 different human sequence antibodies that specifically bind to human CTLA-4.

The invention also provides human sequence antibodies that specifically bind to human CTLA-4 and which block binding of human CTLA-4 to human B7 or do not block binding of human CTLA-4 to human B7.

The invention also provides human sequence antibodies that bind to human CTLA-4 with an equilibrium association constant (Ka) of at least 10^8 M^{-1}. Also provided are human sequence antibodies that bind to human CTLA-4 with an equilibrium association constant (Ka) of at least 10^9 M^{-1}.

The invention also provides human sequence antibodies that specifically bind to human CTLA-4 that block binding of human CTLA-4 to human B7 by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99%, or 100%.

The invention also provides human sequence antibodies that specifically bind to human CTLA-4 having an antibody heavy chain of either IgG or IgM. The IgG

antibody heavy chain can be IgG1, IgG2, IgG3 or IgG4. The invention also provides human sequence antibodies wherein the antibody light chain is a kappa light chain. The human sequence antibody can be encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO:2 through SEQ ID NO:23, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO:16 and SEQ ID NO:6, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO:18 and SEQ ID NO:8, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO:22 and SEQ ID NO:12, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by heavy chain and light chain variable region amino acid sequences as set for the in SEQ ID NO:17 and SEQ ID NO:7, respectively.

The invention provides a human sequence antibody wherein the human sequence antibody is encoded by heavy chain and light chain variable region amino acid sequences as set for the in SEQ ID NO:19 and SEQ ID NO:9, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by heavy chain and light chain variable region amino acid sequences as set for the in SEQ ID NO:23 and SEQ ID NO:13, respectively.

The invention provides a human sequence antibody wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising variable heavy and light chain sequences from V gene segments VH 3-30.3 and VK A-27, respectively.

The invention also provides a human sequence antibody wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising variable heavy and light chain sequences from V gene segments VH 3-33 and VK L-15, respectively.

Some human sequence antibodies of the invention comprise heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGNNKYYADSVKG (SEQ ID NO:32) and TGWLGPFDY (SEQ ID NO:37), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVGSSYLA (SEQ ID NO:24), GAFSRAT (SEQ ID NO:29), and QQYGSSPWT (SEQ ID NO:35), respectively.

Some human sequence antibodies of the invention comprise heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGSNKHYADSVKG (SEQ ID NO:33) and TGWLGPFDY (SEQ ID NO:38), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVSSSFLA (SEQ ID NO:25), GASSRAT (SEQ ID NO:30), and QQYGSSPWT (SEQ ID NO:35), respectively.

Other human sequence antibodies of the invention comprise heavy chain CDR1, CDR2, and CDR3 sequences, SYGMH (SEQ ID NO:28), VIWYDGSNKYYADSVKG (SEQ ID NO:34) and APNYIGAFDV (SEQ ID NO:39), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQGISSWLA (SEQ ID NO:26), AASSLQS (SEQ ID NO:31), and QQYNSYPPT (SEQ ID NO:36), respectively.

The invention also provides human sequence antibodies that specifically bind to human CTLA-4, wherein said human sequence antibody is produced by a transgenic non-human animal. The transgenic non-human animal can be a mouse.

The invention also provides a human sequence antibody that specifically bind to human CTLA-4 that is a Fab fragment.

The invention provides a polyvalent complex comprising at least two human sequence antibodies each of which specifically binds to human CTLA-4. The two different antibodies can be linked to each other covalently or non-covalently.

The invention provides a nucleic acid encoding a heavy chain of a human sequence antibody. The nucleic acid can comprise a nucleotide sequence as set forth in SEQ ID NO:1.

The invention provides a transgenic non-human animal having a genome comprising a human sequence heavy chain transgene and a human sequence light chain transgene, which animal has been immunized with a human CTLA-4, or a fragment or an analog thereof, whereby the animal expresses human sequence antibodies to the human

CTLA-4. The transgenic non-human animal can be a transgenic mouse. The transgenic mouse can comprise HCo7 or HCo12.

The invention provides a hybridoma cell line comprising a B cell obtained from a transgenic non-human animal having a genome comprising a human sequence heavy chain transgene and a human sequence light chain transgene, wherein the hybridoma produces a human sequence antibody that specifically binds to human CTLA-4. In a related embodiment,the hybridoma secretes a human sequence antibody that specifically binds human CTLA-4 or binding fragment thereof, wherein the antibody is selected from the group consisting of: a human sequence antibody comprising heavy chain heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGNNKYYADSVKG (SEQ ID NO:32) and TGWLGPFDY (SEQ ID NO:37), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVGSSYLA (SEQ ID NO:24), GAFSRAT (SEQ ID NO:29), and QQYGSSPWT (SEQ ID NO:35), respectively, and heavy chain and light chain variable region amino acid sequences as set forth in SEQ ID NO:17 and SEQ ID NO:7, respectively; a human sequence antibody comprising heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGSNKHYADSVKG (SEQ ID NO:33) and TGWLGPFDY (SEQ ID NO:38), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVSSSFLA (SEQ ID NO:25), GASSRAT (SEQ ID NO:30), and QQYGSSPWT (SEQ ID NO:35), respectively, and heavy chain and light chain variable region amino acid sequences as set forth in SEQ ID NO:19 and SEQ ID NO:9, respectively; or a human sequence antibody of claim 1, comprising heavy chain CDR1, CDR2, and CDR3 sequences, SYGMH (SEQ ID NO:28), VIWYDGSNKYYADSVKG (SEQ ID NO:34) and APNYIGAFDV (SEQ ID NO:39), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQGISSWLA (SEQ ID NO:26), AASSLQS (SEQ ID NO:31), and QQYNSYPPT (SEQ ID NO:36), respectively, and heavy chain and light chain variable region amino acid sequences as set forth in SEQ ID NO:23 and SEQ ID NO:13, respectively.

The invention provides a pharmaceutical composition comprising a human sequence antibody that specifically binds to human CTLA-4 and a pharmaceutically acceptable carrier. The pharmaceutical composition can further comprise an agent effective to induce an immune response against a target antigen. Also provided are chemotherapeutic agents. In addition, antibodies to immunosuppressive molecules are also provided.

The invention provides a method for inducing, augmenting or prolonging an immune response to an antigen in a patient, comprising administering to the patient an effective dosage of a human sequence antibody that specifically binds to human CTLA-4, wherein the antibody blocks binding of human CTLA-4 to human B7. The antigen can be a tumor antigen, or the antigen can be from a pathogen. The tumor antigen can also be telomerase. The pathogen can be a virus, a bacterium, a fungus or a parasite. The pathogen can also be an HIV. This method can further comprise administering the antigen, or a fragment or an analog thereof, to the patient, whereby the antigen in combination with the human sequence antibody induces, augments or prolongs the immune response. The antigen can be a tumor antigen or a component of an amyloid formation in the patient, such as a patient suffering from Alzheimer's disease and the antigen is AB peptide. This method can further comprise administering a cytokine to the patient.

The invention provides a method of suppressing an immune response in a patient, comprising administering to the patient an effective dosage of a polyvalent preparation comprising at least two human sequence antibodies to human CTLA-4 linked to each other. The invention also provides a method of suppressing an immune response in a patient, comprising administering to the patient an effective dosage of a polyclonal preparation comprising at least two human sequence antibodies to human CTLA-4.

The present invention further provides isolated or recombinant human sequence antibodies and human monoclonal antibodies which specifically bind to human CTLA-4, as well as compositions containing one or a combination of such antibodies. Some of the human sequence antibodies of the invention are characterized by binding to human CTLA-4 with high affinity, and/or by blocking the interaction of human CTLA-4 with its ligand, the human B7-1 and B7-2 molecules. Accordingly, the human sequence antibodies and the human monoclonal antibodies of the invention can be used as diagnostic or therapeutic agents *in vivo* and *in vitro*.

The human sequence antibodies of the invention can encompass various antibody isotypes, or mixtures thereof, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, and IgE. Typically, they include IgG1 (*e.g.*, IgG1k) and IgM isotypes. The human sequence antibodies can be full-length (*e.g.*, an IgG1 or IgG4 antibody) or can include only an antigen-binding portion (*e.g.*, a Fab, F(ab')2, Fv or a single chain Fv fragment). Some human sequence antibodies are recombinant human

sequence antibodies. Some human sequence antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene. The hybridoma can be made by, *e.g.*, fusing the B cell to an immortalized cell. Some human sequence antibodies of the invention are produced by hybridomas referred to as 4C8, 4E10, 4E10.5, 5A8, 5C4, 5C4.1.3, 5D7, 5D7.1, 5E10, 5E10.12, 5G1, 5G1.4, 6A10, 6C9, 6C9.6, 6D9, 6D9.7, 6G4, 7E4, 7E4.4, 7E6, 7H8, 8E8, 8E8.4, 8F8, 8F8.19, 8H1, 9810, 9A10.1, 9B9, 9C1, 9G5, 105B, 10B5.8, 10B9, 10B9.2, 10D1, 10D1.3, 10E11, 10E4, 10E4.5, 11B4, 11D10, 11E4, 11E4.1, 11E8, 11F10, 11F11, 11F9, 11G1, 11G1.5, 1C7, 1H8.8, 2A7, 2A7.6, 2E2, 2E2.7, 2E7, 2E7.2, 2G1, 2G1.2, 3C12, 3E10, 3E10.5, 3E6, 3E6.0, 3F10, 4A1, 4B6 and 4B6.12. Suffixes after the decimal point indicate different clonal isolates of the same hybridoma cell lines.

Some human sequence anti-CTLA-4 antibodies of the present invention can be characterized by one or more of the following properties: a) specificity for human CTLA-4 (specifically binding to human CTLA-4); b) a binding affinity to human CTLA-4 with an equilibrium association constant (K_a) of at least about $10^7\ M^{-1}$, or about $10^9\ M^{-1}$, or about $10^{10}\ M^{-1}$ to $10^{11}\ M^{-1}$ or higher; c) a kinetic association constant (k_a) of at least about 10^3, about 10^4, or about $10^5\ m^{-1}s^{-1}$; and/or, d) a kinetic disassociation constant (k_d) of at least about 10^3, about 10^4, or about $10^5\ m^{-1}s^{-1}$.

In another aspect, the invention provides nucleic acid molecules encoding the human sequence antibodies, or antigen-binding portions, of the invention. Accordingly, recombinant expression vectors that include the antibody-encoding nucleic acids of the invention, and host cells transfected with such vectors, are also encompassed by the invention, as are methods of making the antibodies of the invention by culturing these host cells.

In yet another aspect, the invention provides isolated B-cells from a transgenic non-human animal, *e.g.*, a transgenic mouse, which are capable of expressing various isotypes (*e.g.*, IgG, IgA and/or IgM) of human monoclonal antibodies that specifically bind to human CTLA-4. The isolated B cells can be obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, which has been immunized with a purified or enriched preparation of human CTLA-4 antigen (or antigenic fragment thereof) and/or cells expressing human CTLA-4. The transgenic non-human animal, *e.g.*, a transgenic mouse, can have a genome comprising a human heavy chain transgene and a

human light chain transgene. The isolated B-cells can be immortalized to provide a source (*e.g.*, a hybridoma) of human monoclonal antibodies to human CTLA-4.

Accordingly, the present invention also provides a hybridoma capable of producing human monoclonal antibodies that specifically bind to human CTLA-4. The hybridoma can include a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene fused to an immortalized cell. The transgenic non-human animal can be immunized with a purified or enriched preparation of human CTLA-4 antigen and/or cells expressing human CTLA-4 to generate antibody-producing hybridomas.

In yet another aspect, the invention provides a transgenic non-human animal, such as a transgenic mouse, which express human monoclonal antibodies (also referred to herein as a "HuMAb-Mouse™") that specifically bind to human CTLA-4. The transgenic non-human animal can be a transgenic mouse having a genome comprising a human heavy chain transgene and a human light chain transgene. The transgenic non-human animal can be immunized with a purified or enriched preparation of CTLA-4 antigen (or antigenic fragment thereof) and/or cells expressing the human CTLA-4. The transgenic non-human animal, *e.g.*, the transgenic mouse, can be capable of producing multiple isotypes of human monoclonal antibodies to human CTLA-4 (*e.g.*, IgG, IgA and/or IgM) by undergoing V-D-J recombination and isotype switching. Isotype switching may occur by, *e.g.*, classical or non-classical isotype switching.

In another aspect, the present invention provides methods for producing human sequence antibodies and human sequence monoclonal antibodies that specifically react with human CTLA-4. Some methods of the invention include immunizing a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a human light chain transgene, with a purified or enriched preparation of human CTLA-4 antigen and/or cells expressing human CTLA-4. B cells (*e.g.*, splenic B cells) of the animal can then be obtained and fused with myeloma cells to form immortal, hybridoma cells that secrete human monoclonal antibodies against human CTLA-4.

Anti- human CTLA-4 human monoclonal antibodies of the invention, or antigen binding portions thereof (*e.g.*, Fab), can be derivatized or linked to another functional molecule, *e.g.*, another peptide or protein (*e.g.*, an Fab' fragment). For

example, an antibody or antigen-binding portion of the invention can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities. For example, the human sequence anti-CTLA-4 antibody, or antigen binding fragment thereof, can be conjugated to a therapeutic moiety, *e.g.*, a cytotoxic drug, an enzymatically active toxin, or a fragment thereof, a radioisotope, or a small molecule anti-cancer drug. The antibodies of the invention can also be conjugated to cytotoxic pharmaceuticals, *e.g.*, radiolabeled with a cytotoxic agents, such as, *e.g.*, ^{131}I (*e.g.*, Shen (1997) Cancer 80(12 Suppl):2553-2557), copper-67 (*e.g.*, Deshpande (1988) J. Nucl. Med. 29:217-225) or, *e.g.*, conjugation to the ribosome inactivating protein gelonin (*e.g.*, Boyle (1996) J. of Immunol. 18:221-230).

In another aspect, the present invention provides compositions, *e.g.*, pharmaceutical and diagnostic compositions, comprising a pharmaceutically acceptable carrier and at least one human monoclonal antibody of the invention, or an antigen-binding portion thereof, which specifically binds to human CTLA-4. Some compositions comprise a combination of the human sequence antibodies or antigen-binding portions thereof, preferably each of which binds to a distinct epitope. Compositions, *e.g.*, pharmaceutical compositions, comprising a combination of at least one human sequence antibodies or at least one human monoclonal antibody of the invention, or antigen-binding portions thereof, and at least one bispecific or multispecific molecule of the invention, are also within the scope of the invention.

For *in vivo* methods, the antibody, or antigen-binding portion thereof (or a bispecific or multispecific molecule of the invention), can be administered to a human subject suffering from a T-cell-related disease, or a disease that can be ameliorated or prevented by augmenting or suppressing or prolonging an immune response.

Human sequence monoclonal antibody and human sequence antibody compositions of the invention also can be administered in combination with other known therapies, *e.g.*, an anti-cancer therapy. Accordingly, the invention provides a method for treating cancer in a subject comprising administering a therapeutically effective amount of a pharmaceutical composition of a human sequence antibody together with a pharmaceutical carrier to the subject. Some such methods include a vaccine. Some such vaccines include a tumor cell vaccine, a GM-CSF-modified tumor cell vaccine, or an antigen-loaded dendritic cell vaccine. In some such methods, the cancer is prostate cancer, melanoma, or epithelial cancer.

Human sequence antibodies to human CTLA-4 can be used in methods of treatment requiring either stimulation of immune responses or suppression. The former indication is treated using antibodies that block binding of human CTLA-4 to human B7. Diseases amenable to treatment by stimulation, augmentation of prolonging of immune responses including cancer, including cancers of the prostate, kidney or colon, pathogenic infections, diseases associated with auto-antigens, *e.g.*, amyloidogenic diseases, including Alzheimer's disease, and diseases with inflammatory or allergic components. Immunosuppression is achieved using a polyvalent preparation comprising at least two different antibodies to human CTLA-4 that are linked to each other. Diseases amenable to treatment include graft versus host disease, host versus graft disease, autoimmune diseases and inflammation.

In yet another aspect, the present invention provides a method for detecting *in vitro* or *in vivo* the presence of human CTLA-4 antigen in a sample, *e.g.*, for diagnosing a human CTLA-4-related disease. In some methods, this is achieved by contacting a sample to be tested, along with a control sample, with a human sequence antibody or a human monoclonal antibody of the invention, or an antigen-binding portion thereof (or a bispecific or multispecific molecule), under conditions that allow for formation of a complex between the antibody and human CTLA-4. Complex formation is then detected (*e.g.*; using an ELISA) in both samples, and any statistically significant difference in the formation of complexes between the samples is indicative the presence of human CTLA-4 antigen in the test sample.

A further understanding of the nature and advantages of the present invention may be realized by reference to the remaining portions of the specification, the figures and claims.

All publications, figures, GenBank Accession references (sequences), ATCC Deposits, patents and patent applications cited herein are hereby expressly incorporated by reference for all purposes to the same extent as if each was so individually denoted.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows schematics illustrating the targeted insertion of a neo cassette into the Sma I site of the μ1 exon. Fig. 1A) Schematic diagram of the genomic structure of the μ locus. The filled boxes represent the μ exons; Fig. 1B) Schematic

diagram of the CmD targeting vector. The dotted lines denotes those genomic μ sequences included in the construct. Plasmid sequences are not shown; Fig. 1C) Schematic diagram of the targeted μ locus in which the neo cassette has been inserted into μ1. The box at the lower right shows those RFLP's diagnostic of homologous recombination between the targeting construct and the μ locus. The RFLP's were detected by Southern blot hybridization using probe A, the 915 bp Sac I fragment is shown in Fig. 1C.

Figure 2 shows the results of experiments demonstrating that soluble human sequence antibodies against human CTLA-4 inhibit the binding of recombinant soluble human CTLA-4 to cells expressing mouse B7.1, as described in detail, below.

Figure 3 shows the results of a competitive binding assay to identify human sequence antibodies of the invention that recognize non-overlapping epitopes on human CTLA-4, as described in detail, below.

Figure 4 shows preliminary nucleotide sequence data for the heavy and light chain fragment of the anti-CTLA-4 antibody 10D1.3.

Figure 5 shows the nucleotide sequences of the light chain variable Regions (V_K) of Anti-Human CTLA-4 Antibodies. The anti-CTLA-4 antibodies 10D1 (SEQ ID NO:6) and 4B6 (SEQ ID NO:8) derived from the V_K A-27 germline sequence (SEQ ID NO:4) are depicted at the top of the Figure. The anti-CTLA-4 antibody 1E2 (SEQ ID NO:12) derived from the V_K L-15 germline sequence (SEQ ID NO:10) is shown at the bottom of the Figure. The V_K sequences of three anti-CTLA-4 antibodies are aligned with their germline encoded V_K gene sequences. The complementary determining residues (CDR) are labeled. Dashes denote sequence identity.

Figure 6 shows the nucleotide sequences of the heavy chain variable Regions (V_H) of Anti-Human CTLA-4 Antibodies. The anti-CTLA-4 antibodies 10D1 (SEQ ID NO:16) and 4B6 (SEQ ID NO:18) derived from the V_H 3-30.3 germline sequence (SEQ ID NO:14) are depicted at the top of the Figure. The anti-CTLA-4 antibody 1E2 (SEQ ID NO:22) derived from the V_H 3-33 germline sequence (SEQ ID NO:20) is shown at the bottom of the Figure. The V_H sequences of three anti-CTLA-4 antibodies are aligned with their germline encoded sequences. The complementary determining residues (CDR) are labeled. Dashes denote sequence identity.

Figure 7 shows the predicted amino acid sequences of the light chain Variable Regions of Anti-Human CTLA-4 Antibodies. The predicted amino acid V_K

sequences of the anti-CTLA-4 antibodies described in **Figure 5** are shown. The anti-CTLA-4 antibodies 10D1 (SEQ ID NO:7) and 4B6 (SEQ ID NO:9) derived from the V_K A-27 germline sequence (SEQ ID NO:5) are depicted at the top of the Figure. The anti-CTLA-4 antibody 1E2 (SEQ ID NO:13) derived from the V_K L-15 germline sequence (SEQ ID NO:11) is shown at the bottom of the Figure.

Figure 8 shows the predicted amino acid sequences of the heavy chain Variable Regions of Anti-Human CTLA-4 Antibodies. The predicted amino acid V_H sequences of the anti-CTLA-4 antibodies described in **Figure 6** are shown. The anti-CTLA-4 antibodies 10D1 (SEQ ID NO:17) and 4B6 (SEQ ID NO:19) derived from the V_H 3-30.3 germline sequence (SEQ ID NO:15) are depicted at the top of the Figure. The anti-CTLA-4 antibody 1E2 (SEQ ID NO:23) derived from the V_H 3-33 germline sequence (SEQ ID NO:21) is shown at the bottom of the Figure.

Figure 9 shows the results of binding experiments of MAb 10D1 to recombinant human CTLA-4 by ELISA. MAb 10D1 binds with dose-dependent and saturating kinetics to purified recombinant CTLA-4.

Figure 10 shows the binding of 10D1 to a CTLA4-expressing T-cell line. These data show that MAb 10D1 binds with dose-dependent and saturating kinetics to cells expressing CTLA-4.

Figure 11 shows inhibition of binding of human B7.2 Ig to CTLA4-expressing T-cells. These data show that MAb 10D1 can efficiently block B7.2 binding to CTLA-4 as compared to a control human MAb.

Figure 12 shows the results for blocking CTLA4-FITC binding to murine B7.1-expressing cells. These data show that MAb 10D1 can efficiently block CTLA-4 binding to B7.1 as compared to a control human MAb.

Figure 13 shows competitive ELISAs of anti-CTLA-4 human MAbs demonstrating epitope group classifications.

Figure 14 shows CTLA-4 expression on PHA-stimulated T-cells. Activated, but not resting T cells, express low but detectable levels of CTLA-4 at the cell surface.

Figure 15 shows the results of MAb 10D1 in Complement Dependent Lysis of Activated T Cells. No lysis of PHA-activated T cells is observed.

Figure 16 shows the results of MAb 10D1 in Antibody-Dependent Lysis of Activated T Cells. No lysis of PHA-activated T cells is observed with 10D1 and mononuclear cells.

Figure 17 shows anti-10D1 IgM and IgG responses in cynomolgus monkeys injected with 10D1 antibody. No significant antibody response to 10D1 is observed.

DETAILED DESCRIPTION

The present invention provides novel antibody-based therapies for treating and diagnosing diseases characterized by expression, particularly over-expression, or activation of, particularly overactivation, of human CTLA-4 and/or related molecules. Therapies of the invention employ human sequence antibodies, human sequence monoclonal antibodies, or antigen-binding portions thereof, which bind to an epitope present on human CTLA-4. These human sequence anti-CTLA-4 antibodies can act as functional antagonists (*e.g.*, inhibiting the ability of CTLA-4 to bind ligand or to activate the cell, *e.g.*, by inhibiting its ability to transmit a signal to the cell) or agonists (*e.g.*, to simulate the effect of ligand).

The human sequence antibodies of the invention can be produced in a non-human transgenic animal, *e.g.*, a transgenic mouse, capable of producing multiple isotypes of human (*e.g.*, monoclonal or polyclonal) antibodies to human CTLA-4 (*e.g.*, IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching. Accordingly, various aspects of the invention include antibodies and antibody fragments, and pharmaceutical compositions thereof, as well as non-human transgenic animals, and B-cells and hybridomas for making such monoclonal antibodies. Methods of using the antibodies of the invention to detect a cell expressing human CTLA-4 or a related, cross-reactive growth factor receptor, or to inhibit growth, differentiation and/or motility of a cell expressing human CTLA-4, either *in vitro* or *in vivo*, are also encompassed by the invention.

Except when noted, the terms "patient" or "subject" are used interchangeably and refer to mammals such as human patients and non-human primates, as well as experimental animals such as rabbits, rats, and mice, and other animals.

The term "treating" includes the administration of the compounds or agents of the present invention to prevent or delay the onset of the symptoms, complications, or biochemical indicia of a disease, alleviating the symptoms or arresting or inhibiting further development of the disease, condition, or disorder (*e.g.*, autoimmune disease). Treatment may be prophylactic (to prevent or delay the onset of the disease, or

to prevent the manifestation of clinical or subclinical symptoms thereof) or therapeutic suppression or alleviation of symptoms after the manifestation of the disease.

In general, the phrase "well tolerated" refers to the absence of adverse changes in health status that occur as a result of the treatment and would affect treatment decisions.

The term "lymphocyte" as used herein has the normal meaning in the art, and refers to any of the mononuclear, nonphagocytic leukocytes, found in the blood, lymph, and lymphoid tissues, *i.e.*, B and T lymphocytes.

The phrase "subpopulations of T lymphocytes" or "T cell subset(s)" refers to T lymphocytes or T cells characterized by the expression of particular cell surface markers (*see* Barclay, A. N. *et al.*. (eds.), 1997, The Leukocyte Antigen Facts Book, 2nd. edition, Academic Press, London, United Kingdom). The term "stable" in reference to T cells refers to the fact that the frequency or percentage of a T cell subset does not change over the course or duration of the administration of an agent.

The terms "cytotoxic T lymphocyte-associated antigen-4," "CTLA-4," "CTLA4," "CTLA-4 antigen" and "CD152" (*see, e.g.*, Murata (1999) Am. J. Pathol. 155:453-460) are used interchangeably, and include variants, isoforms, species homologs of human CTLA-4, and analogs having at least one common epitope with CTLA-4 (*see, e.g.*, Balzano (1992) Int. J. Cancer Suppl. 7:28-32).

The complete cDNA sequence of human CTLA-4 has the Genbank accession number L15006. The region of amino acids 1-37 is the leader peptide; 38-161 is the extracellular V-like domain; 162-187 is the transmembrane domain; and 188-223 is the cytoplasmic domain. Variants of the nucleotide sequence have been reported, including a G to A transition at position 49, a C to T transition at position 272, and an A to G transition at position 439. The complete DNA sequence of mouse CTLA-4 has the EMBL accession number X05719 (Brunet *et al.* (1987) Nature 328:267-270). The region of amino acids 1-35 is the leader peptide.

The complete DNA sequence of human B7-1 (CD80) has the Genbank accession number X60958; the accession number for the mouse sequence is X60958; the accession number for the rat sequence is U05593. The complete cDNA sequence of human B7-2 (CD86) has the Genbank accession number L25259; the accession number for the mouse sequence is L25606.

The genes encoding CD28 have been extensively characterized. The chicken mRNA sequence has the Genbank accession number X67915. The rat mRNA

sequence has the Genbank accession number X55288. The human mRNA sequence has the Genbank accession number J02988. The mouse mRNA sequence has the Genbank accession number M34536.

The term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

An intact "antibody" comprises at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system. The term antibody includes antigen-binding portions of an intact antibody that retain capacity to bind CTLA-4. Examples of binding include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al..*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as

a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); *See, e.g.*, Bird *et al.*. (1988) *Science* 242:423-426; and Huston *et al.*. (1988) *Proc. Natl. Acad. Sci.* USA 85:5879-5883). Such single chain antibodies are included by reference to the term "antibody" Fragments can be prepared by recombinant techniques or enzymatic or chemical cleavage of intact antibodies.

A bispecific antibody has two different binding specificities, see. *e.g.*, U.S. Patents. 5,922,845 and 5,837,243; Zeilder (1999) J. Immunol. 163:1246-1252; Somasundaram (1999) Hum. Antibodies 9:47-54; Keler (1997) Cancer Res. 57:4008-4014. For example, the invention provides bispecific antibodies having one binding site for a cell surface antigen, such as human CTLA-4, and a second binding site for an Fc receptor on the surface of an effector cell. The invention also provides multispecific antibodies, which have at least three binding sites. The term "bispecific antibodies" further includes diabodies. Diabodies are bivalent, bispecific antibodies in which the VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (*See, e.g.*, Holliger, P., *et al.*. (1993) *Proc. Natl. Acad. Sci.* USA 90:6444-6448; Poljak, R.J., *et al.*. (1994) Structure 2:1121-1123).

The term "human sequence antibody" includes antibodies having variable and constant regions (if present) derived from human germline immunoglobulin sequences. The human sequence antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human sequence antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences (*i.e.*, humanized antibodies).

The terms "monoclonal antibody" or "monoclonal antibody composition" refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions (if present) derived from human germline immunoglobulin sequences. In one

embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term "diclonal antibody" refers to a preparation of at least two antibodies to human CTLA-4. Typically, the different antibodies bind different epitopes.

The term "oligoclonal antibody" refers to a preparation of 3 to 100 different antibodies to human CTLA-4. Typically, the antibodies in such a preparation bind to a range of different epitopes.

The term "polyclonal antibody" refers to a preparation of more than 1 (two or more) different antibodies to human CTLA-4. Such a preparation includes antibodies binding to a range of different epitopes.

The invention provides human sequence antibodies to human CTLA-4 which block or antagonize signals transduced by the human CTLA-4 receptor. Some of these antibodies can bind to an epitope on human CTLA-4 so as to inhibit CTLA-4 from interacting with a human B7 counterreceptor. Because interaction of human CTLA-4 with human B7 transduces a signal leading to inactivation of T-cells bearing the human CTLA-4 receptor, antagonism of the interaction effectively induces, augments or prolongs the activation of T cells bearing the human CTLA-4 receptor, thereby prolonging or augmenting an immune response. A "blocking antibody" refers to an antibody that reduces the binding of soluble human CTLA-4 to cell-expressed human B7 ligand by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99% or 99.9% under conditions in which the ratio of antibody combining site to human CTLA-4 ligand binding site is greater than 1:1 and the concentration of antibody is greater than 10^{-8} M.

Other antibody preparations, sometimes referred to as multivalent preparations, bind to human CTLA-4 in such a manner as to crosslink multiple human CTLA-4 receptors on the same cell. Cross-linking of receptor has the same or similar effect to binding of human CTLA-4 to human B7. Thus, cross-linking of receptors effectively agonizes the human CTLA-4 response resulting in immunosuppression.

Cross-linking can also be accomplished by combining soluble divalent antibodies having different epitope specificities. These polyclonal antibody preparations comprise at least two pairs of heavy and light chains binding to different epitopes on human CTLA-4 such that an immunosuppressing signal can be transduced as a result of human CTLA-4 crosslinking.

The term "recombinant human antibody" includes all human sequence antibodies of the invention that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (described further in Section I, below); antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions (if present) derived from human germline immunoglobulin sequences. Such antibodies can, however, be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

A "heterologous antibody" is defined in relation to the transgenic non-human organism producing such an antibody. This term refers to an antibody having an amino acid sequence or an encoding nucleic acid sequence corresponding to that found in an organism not consisting of the transgenic non-human animal, and generally from a species other than that of the transgenic non-human animal.

A "heterohybrid antibody" refers to an antibody having a light and heavy chains of different organismal origins. For example, an antibody having a human heavy chain associated with a murine light chain is a heterohybrid antibody. Examples of heterohybrid antibodies include chimeric and humanized antibodies, discussed *supra*.

The term "substantially pure" or "isolated" means an object species (*e.g.*, an antibody of the invention) has been identified and separated and/or recovered from a component of its natural environment such that the object species is the predominant species present (*i.e.*, on a molar basis it is more abundant than any other individual species in the composition); a "substantially pure" or "isolated" composition also means where the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. A substantially pure or isolated composition can also comprise more than about 80 to 90 percent by weight of all macromolecular species present in the composition. An isolated object species (*e.g.*, antibodies of the invention) can also be purified to essential homogeneity (contaminant species cannot be detected in

the composition by conventional detection methods) wherein the composition consists essentially of derivatives of a single macromolecular species. An isolated antibody to human CTLA-4 can be substantially free of other antibodies that lack binding to human CTLA-4 and bind to a different antigen. An isolated antibody that specifically binds to an epitope, isoform or variant of human CTLA-4 may, however, have cross-reactivity to other related antigens, *e.g.*, from other species (*e.g.*, CTLA-4 species homologs). Moreover, an isolated antibody of the invention be substantially free of other cellular material (*e.g.*, non-immunoglobulin associated proteins) and/or chemicals.

"Specific binding'' refers to antibody binding to a predetermined antigen. The phrase "specifically (or selectively) binds" to an antibody refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics.Typically, the antibody binds with an association constant (K_a) of at least about 1 x 10^6 M^{-1} or 10^7 M^{-1}, or about 10^8 M^{-1} to 10^9 M^{-1}, or about 10^{10} M^{-1} to 10^{11} M^{-1} or higher, and binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (*e.g.*, BSA, casein) other than the predetermined antigen or a closely-related antigen. The phrases "an antibody recognizing an antigen" and " an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen".

The phrase "specifically bind(s)" or "bind(s) specifically" when referring to a peptide refers to a peptide molecule which has intermediate or high binding affinity, exclusively or predominately, to a target molecule. The phrases "specifically binds to" refers to a binding reaction which is determinative of the presence of a target protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated assay conditions, the specified binding moieties bind preferentially to a particular target protein and do not bind in a significant amount to other components present in a test sample. Specific binding to a target protein under such conditions may require a binding moiety that is selected for its specificity for a particular target antigen. A variety of assay formats may be used to select ligands that are specifically reactive with a particular protein. For example, solid-phase ELISA immunoassays, immunoprecipitation, Biacore and Western blot are used to identify peptides that specifically react with CTLA-4. Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 times background.

The term "high affinity" for an IgG antibody refers to an equilibrium association constant (K_a) of at least about 10^7M^{-1}, at least about 10^8M^{-1}, at least about 10^9M^{-1}, at least about $10^{10}M^{-1}$, at least about $10^{11}M^{-1}$, or at least about $10^{12}M^{-1}$ or greater, *e.g.*, up to $10^{13}M^{-1}$ or $10^{14}M^{-1}$ or greater. However, "high affinity" binding can vary for other antibody isotypes.

The term "K_a", as used herein, is intended to refer to the equilibrium association constant of a particular antibody-antigen interaction. This constant has units of 1/M.

The term "K_d", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction. This constant has units of M.

The term "k_a", as used herein, is intended to refer to the kinetic association constant of a particular antibody-antigen interaction. This constant has units of 1/Ms

The term "k_d", as used herein, is intended to refer to the kinetic dissociation constant of a particular antibody-antigen interaction. This constant has units of 1/s.

"Particular antibody-antigen interactions" refers to the experimental conditions under which the equilibrium and kinetic constants are measured.

"Isotype" refers to the antibody class (*e.g.*, IgM or IgGl) that is encoded by heavy chain constant region genes.

"Isotype switching" refers to the phenomenon by which the class, or isotype, of an antibody changes from one Ig class to one of the other Ig classes.

"Nonswitched isotype" refers to the isotypic class of heavy chain that is produced when no isotype switching has taken place; the CH gene encoding the nonswitched isotype is typically the first CH gene immediately downstream from the functionally rearranged VDJ gene. Isotype switching has been classified as classical or non-classical isotype switching. Classical isotype switching occurs by recombination events which involve at least one switch sequence region in the transgene. Non-classical isotype switching may occur by, for example, homologous recombination between human σ_μ and human Σ_μ (δ-associated deletion). Alternative non-classical switching mechanisms, such as intertransgene and/or interchromosomal recombination, among others, may occur and effectuate isotype switching.

The term "switch sequence" refers to those DNA sequences responsible for switch recombination. A "switch donor" sequence, typically a μ switch region, are 5' (*i.e.*, upstream) of the construct region to be deleted during the switch recombination. The "switch acceptor" region are between the construct region to be deleted and the replacement constant region (*e.g.*, γ, ε, etc.). As there is no specific site where recombination always occurs, the final gene sequence is not typically predictable from the construct.

"Glycosylation pattern" is defined as the pattern of carbohydrate units that are covalently attached to a protein, more specifically to an immunoglobulin protein. A glycosylation pattern of a heterologous antibody can be characterized as being substantially similar to glycosylation patterns which occur naturally on antibodies produced by the species of the non-human transgenic animal, when one of ordinary skill in the art would recognize the glycosylation pattern of the heterologous antibody as being more similar to said pattern of glycosylation in the species of the non-human transgenic animal than to the species from which the CH genes of the transgene were derived.

The term "naturally-occurring'' as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "rearranged" refers to a configuration of a heavy chain or light chain immunoglobulin locus wherein a V segment is positioned immediately adjacent to a D-J or J segment in a conformation encoding essentially a complete VH or VL domain, respectively. A rearranged immunoglobulin gene locus can be identified by comparison to germline DNA; a rearranged locus has at least one recombined heptamer/nonamer homology element.

The term "unrearranged" or "germline configuration" in reference to a V segment refers to the configuration wherein the V segment is not recombined so as to be immediately adjacent to a D or J segment.

The term "nucleic acid" is intended to include DNA molecules and RNA molecules. A nucleic acid can be single-stranded or double-stranded.

The term "isolated nucleic acid" in reference to nucleic acids encoding antibodies or antibody portions (*e.g.*, VH, VL, CDR3) that bind to CTLA-4, is intended to

refer to a nucleic acid in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than CTLA-4, which other sequences may naturally flank the nucleic acid in human genomic DNA. SEQ ID NOs: 4-23 comprise the nucleotide and amino acid sequences comprising the heavy chain (VH) and light chain (VL) variable regions of the 10D1, 4B6 and 1E2 human anti-CTLA-4 monoclonal antibodies of the invention.

The term "substantially identical," in the context of two nucleic acids or polypeptides refers to two or more sequences or subsequences that have at least about 80%, about 90, about 95% or higher nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using the following sequence comparison method and/or by visual inspection. For example, the invention provides nucleic acids having sequences that are substantially identical to SEQ ID NO:1, SEQ ID NO:2. Such "substantially identical" sequences are typically considered to be homologous. The "substantial identity" can exist over a region of sequence that is at least about 50 residues in length, over a region of at least about 100 residues, or over a region at least about 150 residues, or over the full length of the two sequences to be compared. As described below, any two antibody sequences can only be aligned in one way, by using the numbering scheme in Kabat. Therefore, for antibodies, percent identity has a unique and well-defined meaning.

Amino acids from the variable regions of the mature heavy and light chains of immunoglobulins are designated Hx and Lx respectively, where x is a number designating the position of an amino acid according to the scheme of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987 and 1991). Kabat lists many amino acid sequences for antibodies for each subgroup, and lists the most commonly occurring amino acid for each residue position in that subgroup to generate a consensus sequence. Kabat uses a method for assigning a residue number to each amino acid in a listed sequence, and this method for assigning residue numbers has become standard in the field. Kabat's scheme is extendible to other antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. The use of the Kabat numbering system readily identifies amino acids at equivalent positions in different antibodies. For example, an amino acid at the L50 position of a human antibody occupies the equivalent position to an amino acid position L50 of a mouse antibody. Likewise,

nucleic acids encoding antibody chains are aligned when the amino acid sequences encoded by the respective nucleic acids are aligned according to the Kabat numbering convention.

The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (*e.g.*, total cellular or library DNA or RNA), wherein the particular nucleotide sequence is detected at least at about 10 times background. In one embodiment, a nucleic acid can be determined to be within the scope of the invention (*e.g.*, is substantially identical to SEQ ID NO:1 or SEQ ID NO:2) by its ability to hybridize under stringent conditions to a nucleic acid otherwise determined to be within the scope of the invention (such as the exemplary sequences described herein).

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but not to other sequences in significant amounts (a positive signal (*e.g.*, identification of a nucleic acid of the invention) is about 10 times background hybridization). Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found An extensive guide to the hybridization of nucleic acids is found in *e.g.*, Sambrook, ed., MOLECULAR CLONING: A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY: HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993).

Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes

(*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide as described in Sambrook (cited below). For high stringency hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary high stringency or stringent hybridization conditions include: 50% formamide, 5x SSC and 1% SDS incubated at 42° C or 5x SSC and 1% SDS incubated at 65° C, with a wash in 0.2x SSC and 0.1% SDS at 65° C. . For selective or specific hybridization, a positive signal (*e.g.*, identification of a nucleic acid of the invention) is about 10 times background hybridization. Stringent hybridization conditions that are used to identify nucleic acids within the scope of the invention include, *e.g.*, hybridization in a buffer comprising 50% formamide, 5x SSC, and 1% SDS at 42°C, or hybridization in a buffer comprising 5x SSC and 1% SDS at 65°C, both with a wash of 0.2x SSC and 0.1% SDS at 65°C. In the present invention, genomic DNA or cDNA comprising nucleic acids of the invention can be identified in standard Southern blots under stringent conditions using the nucleic acid sequences disclosed here. Additional stringent conditions for such hybridizations (to identify nucleic acids within the scope of the invention) are those which include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C.

However, the selection of a hybridization format is not critical - it is the stringency of the wash conditions that set forth the conditions which determine whether a nucleic acid is within the scope of the invention. Wash conditions used to identify nucleic acids within the scope of the invention include, *e.g.*: a salt concentration of about 0.02 molar at pH 7 and a temperature of at least about 50°C or about 55°C to about 60°C; or, a salt concentration of about 0.15 M NaCl at 72°C for about 15 minutes; or, a salt concentration of about 0.2X SSC at a temperature of at least about 50°C or about 55°C to about 60°C for about 15 to about 20 minutes; or, the hybridization complex is washed twice with a solution with a salt concentration of about 2X SSC containing 0.1% SDS at room temperature for 15 minutes and then washed twice by 0.1X SSC containing 0.1% SDS at 68°C for 15 minutes; or, equivalent conditions. See Sambrook, Tijssen and Ausubel for a description of SSC buffer and equivalent conditions.

The nucleic acids of the invention be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel

electrophoresis and others well known in the art. *see, e.g.*, Sambrook, Tijssen and Ausubel. The nucleic acid sequences of the invention and other nucleic acids used to practice this invention, whether RNA, cDNA, genomic DNA, or hybrids thereof, may be isolated from a variety of sources, genetically engineered, amplified, and/or expressed recombinantly. Any recombinant expression system can be used, including, in addition to bacterial, *e.g.*, yeast, insect or mammalian systems. Alternatively, these nucleic acids can be chemically synthesized *in vitro*. Techniques for the manipulation of nucleic acids, such as, *e.g.*, subcloning into expression vectors, labeling probes, sequencing, and hybridization are well described in the scientific and patent literature, *see, e.g.*, Sambrook, Tijssen and Ausubel. Nucleic acids can be analyzed and quantified by any of a number of general means well known to those of skill in the art. These include, *e.g.*, analytical biochemical methods such as NMR, spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and hyperdiffusion chromatography, various immunological methods, such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immuno-fluorescent assays, Southern analysis, Northern analysis, dot-blot analysis, gel electrophoresis (*e.g.*, SDS-PAGE), RT-PCR, quantitative PCR, other nucleic acid or target or signal amplification methods, radiolabeling, scintillation counting, and affinity chromatography.

The nucleic acid compositions of the present invention, while often in a native sequence (except for modified restriction sites and the like), from either cDNA, genomic or mixtures may be mutated, thereof in accordance with standard techniques to provide gene sequences. For coding sequences, these mutations, may affect amino acid sequence as desired. In particular, DNA sequences substantially homologous to or derived from native V, D, J, constant, switches and other such sequences described herein are contemplated (where "derived" indicates that a sequence is identical or modified from another sequence).

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. With respect to transcription regulatory sequences, operably linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein coding

regions, contiguous and in reading frame. For switch sequences, operably linked indicates that the sequences are capable of effecting switch recombination.

The term "vector" is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell") refers to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

The term "minilocus transgene" refers to a transgene that comprises a portion of the genomic immunoglobulin locus having at least one internal (*i.e.*, not at a terminus of the portion) deletion of a non-essential DNA portion (*e.g.*, intervening sequence; intron or portion thereof) as compared to the naturally-occurring germline Ig locus.

A "label" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include

^{32}P, fluorescent dyes, electron-dense reagents, enzymes (*e.g.*, as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins for which antisera or monoclonal antibodies are available (*e.g.*, the polypeptides of the invention can be made detectable, *e.g.*, by incorporating a radiolabel into the peptide, and used to detect antibodies specifically reactive with the peptide).

The term "sorting" in the context of cells as used herein to refers to both physical sorting of the cells, as can be accomplished using, *e.g.*, a fluorescence activated cell sorter, as well as to analysis of cells based on expression of cell surface markers, *e.g.*, FACS analysis in the absence of sorting.

The phrase "immune cell response" refers to the response of immune system cells to external or internal stimuli (*e.g.*, antigen, cytokines, chemokines, and other cells) producing biochemical changes in the immune cells that result in immune cell migration, killing of target cells, phagocytosis, production of antibodies, other soluble effectors of the immune response, and the like.

The terms "T lymphocyte response" and "T lymphocyte activity" are used here interchangeably to refer to the component of immune response dependent on T lymphocytes (*i.e.*, the proliferation and/or differentiation of T lymphocytes into helper, cytotoxic killer, or suppressor T lymphocytes, the provision of signals by helper T lymphocytes to B lymphocytes that cause or prevent antibody production, the killing of specific target cells by cytotoxic T lymphocytes, and the release of soluble factors such as cytokines that modulate the function of other immune cells).

The term "immune response" refers to the concerted action of lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

Components of an immune response may be detected in vitro by various methods that are well known to those of ordinary skill in the art. For example, (1) cytotoxic T lymphocytes can be incubated with radioactively labeled target cells and the lysis of these target cells detected by the release of radioactivity, (2) helper T lymphocytes can be incubated with antigens and antigen presenting cells and the synthesis and secretion of cytokines measured by standard methods (Windhagen A; *et*

al.., 1995, Immunity 2(4): 373-80), (3) antigen presenting cells can be incubated with whole protein antigen and the presentation of that antigen on MHC detected by either T lymphocyte activation assays or biophysical methods (Harding *et al..*, 1989, Proc. Natl. Acad. Sci., 86: 4230-4), (4) mast cells can be incubated with reagents that cross-link their Fc-epsilon receptors and histamine release measured by enzyme immunoassay (Siraganian, *et al..*, 1983, TIPS 4: 432-437).

Similarly, products of an immune response in either a model organism (*e.g.*, mouse) or a human patient can also be detected by various methods that are well known to those of ordinary skill in the art. For example, (1) the production of antibodies in response to vaccination can be readily detected by standard methods currently used in clinical laboratories, *e.g.*, an ELISA; (2) the migration of immune cells to sites of inflammation can be detected by scratching the surface of skin and placing a sterile container to capture the migrating cells over scratch site (Peters *et al..*, 1988, Blood 72: 1310-5); (3) the proliferation of peripheral blood mononuclear cells in response to mitogens or mixed lymphocyte reaction can be measured using ^{3}H-thymidine; (4) the phagocitic capacity of granulocytes, macrophages, and other phagocytes in PBMCs can be measured by placing PMBCs in wells together with labeled particles (Peters *et al..*, 1988); and (5) the differentation of immune system cells can be measured by labeling PBMCs with antibodies to CD molecules such as CD4 and CD8 and measuring the fraction of the PBMCs expressing these markers.

As used herein, the phrase "signal transduction pathway" or "signal transduction event" refers to at least one biochemical reaction, but more commonly a series of biochemical reactions, which result from interaction of a cell with a stimulatory compound or agent. Thus, the interaction of a stimulatory compound with a cell generates a "signal" that is transmitted through the signal transduction pathway, ultimately resulting in a cellular response, *e.g.*, an immune response described above.

A signal transduction pathway refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. Signal transduction molecules of the present invention include, for example, MAb 147.1 of the invention. As used herein, the phrase "cell surface receptor" includes molecules and complexes of molecules capable of receiving a signal and the transmission of such a signal across the plasma membrane of a cell. An example of a "cell surface receptor" of the present invention is the T cell receptor (TCR) or the B7 ligands of CTLA-4.

A signal transduction pathway in a cell can be initiated by interaction of a cell with a stimulator that is inside or outside of the cell. If an exterior (*i.e.*, outside of the cell) stimulator (*e.g.*, an MHC-antigen complex on an antigen presenting cell) interacts with a cell surface receptor (*e.g.*, a T cell receptor), a signal transduction pathway can transmit a signal across the cell's membrane, through the cytoplasm of the cell, and in some instances into the nucleus. If an interior (*e.g.*, inside the cell) stimulator interacts with an intracellular signal transduction molecule, a signal transduction pathway can result in transmission of a signal through the cell's cytoplasm, and in some instances into the cell's nucleus.

Signal transduction can occur through, *e.g.*, the phosphorylation of a molecule; non-covalent allosteric interactions; complexing of molecules; the conformational change of a molecule; calcium release; inositol phosphate production; proteolytic cleavage; cyclic nucleotide production and diacylglyceride production. Typically, signal transduction occurs through phosphorylating a signal transduction molecule.

The term "nonspecific T cell activation" refers to the stimulation of T cells independent of their antigenic specificity.

PRODUCTION OF HUMAN ANTIBODIES TO CTLA-4

The monoclonal antibodies (mAbs) and the human sequence antibodies of the invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology *e.g.*, the standard somatic cell hybridization technique of Kohler and Milstein, *Nature* 256: 495 (1975). Any technique for producing monoclonal antibody can be employed *e.g.*, viral or oncogenic transformation of B lymphocytes. One animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known (*see, e.g.*, Harlow and Lane (1988), *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor New York).

Human monoclonal antibodies and human sequence antibodies directed against human CTLA-4 can be generated using transgenic mice carrying a human immune system rather than the mouse system. These transgenic mice, also referred to herein as "HuMAb-Mouse™", contain a human immunoglobulin gene miniloci that

encodes unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg, N. *et al.*. (1994) *Nature* 368(6474): 856-859 and US patent 5,770,429). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal (Lonberg, N. *et al.*. (1994), *supra*; reviewed in Lonberg, N. (1994) H*andbook of Experimental Pharmacology* 113:49-101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* Vol. 13: 65-93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci* 764:536-546). The preparation of transgenic mice is described in detail Section II below and in Taylor, L. *et al.*. (1992) *Nucleic Acids Research* 20:6287-6295; Chen, J. *et al.*. (1993) *International Immunology* 5: 647-656; Tuaillon *et al.*. (1993) *Proc. Natl. Acad. Sci USA* 90:3720-3724; Choi *et al.*. (1993) *Nature Genetics* 4:117-123; Chen, J. *et al.*. (1993) EMBO J. 12: 821-830; Tuaillon *et al.*. (1994) *J. Immunol.* 152:2912-2920; Lonberg *et al.*., (1994) *Nature* 368(6474): 856-859; Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Taylor, L. *et al.*. (1994) *International Immunology* 6: 579-591; Lonberg, N. and Huszar, D. (1995) Intern. Rev. Immunol. Vol. 13: 65-93; Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci* 764:536-546; Fishwild, D. *et al.*. (1996) *Nature Biotechnology* 14: 845-851. *See further*, U.S. Patent Nos. 5,625,126 and 5,770,429, both to Lonberg and Kay, and GenPharm International; U.S. Patent No. 5,545,807 to Surani *et al.*.; International Publication Nos. WO 98/24884, published on June 11, 1998; WO 94/25585, published November 10, 1994; WO 93/1227, published June 24, 1993; WO 92/22645, published December 23, 1992; WO 92/03918, published March 19, 1992. Alternatively, the CMD and HCo12 transgenes, described in Examples 1 and 2, below, can be used to generate human anti-CTLA-4 antibodies.

Detailed procedures to generate fully human monoclonal antibodies to CTLA-4 are described in the Examples below. Cumulative experience with various antigens has shown that the transgenic mice respond when initially immunized intraperitoneally (IP) with antigen in complete Freund's adjuvant, followed by every other week IP immunizations (up to a total of 6) with antigen in incomplete Freund's adjuvant. However, adjuvants other than Freund's are also found to be effective. In addition, whole cells in the absence of adjuvant are found to be highly immunogenic. The immune response can be monitored over the course of the immunization protocol

with plasma samples being obtained by retroorbital bleeds. The plasma can be screened by ELISA (as described below), and mice with sufficient titers of anti-CTLA-4 human immunoglobulin can be used for fusions. Mice can be boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. It is expected that 2-3 fusions for each immunization may need to be performed. Between 6 and 24 mice are typically immunized for each antigen. Usually both HCo7 and HCo12 strains are used. In addition, both HCo7 and HCo12 transgene can be bred together into a single mouse having two different human heavy chain transgenes.

To purify human anti-CTLA-4 antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, NJ). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD280 using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80 °C.

To determine if the selected human anti-CTLA-4 monoclonal antibodies bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, IL). Competition studies using unlabeled monoclonal antibodies and biotinylated monoclonal antibodies can be performed using CTLA-4 coated-ELISA plates as described above. Biotinylated MAb binding can be detected with a strep-avidin-alkaline phosphatase probe.

To determine the isotype of purified antibodies, isotype ELISAs can be performed. Wells of microtiter plates can be coated with 1 μg/ml of anti-human IgG overnight at 4°C. After blocking with 1% BSA, the plates are reacted with 1 μg/ml or less of monoclonal antibodies or purified isotype controls, at ambient temperature for one to two hours. The wells can then be reacted with either human IgG1 or human IgM-specific alkaline phosphatase-conjugated probes. Plates are developed and analyzed as described above.

To demonstrate binding of monoclonal antibodies to live cells expressing the CTLA-4, flow cytometry can be used. Briefly, cell lines expressing CTLA-4 (grown under standard growth conditions) are mixed with various concentrations of monoclonal antibodies in PBS containing 0.1% BSA and 10% fetal calf serum, and incubated at 37°C for 1 hour. After washing, the cells are reacted with Fluorescein-labeled anti-human IgG

antibody under the same conditions as the primary antibody staining. The samples can be analyzed by FACScan instrument using light and side scatter properties to gate on single cells. An alternative assay using fluorescence microscopy may be used (in addition to or instead of) the flow cytometry assay. Cells can be stained exactly as described above and examined by fluorescence microscopy. This method allows visualization of individual cells, but may have diminished sensitivity depending on the density of the antigen.

Anti-CTLA-4 human IgGs can be further tested for reactivity with CTLA-4 antigen by Western blotting. Briefly, cell extracts from cells expressing CTLA-4 can be prepared and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens are transferred to nitrocellulose membranes, blocked with 10% fetal calf serum, and probed with the monoclonal antibodies to be tested. Human IgG binding can be detected using anti-human IgG alkaline phosphatase and developed with BCIP/NBT substrate tablets (Sigma Chem. Co., St. Louis, MO).

PRODUCTION OF TRANSGENIC NON-HUMAN ANIMALS THAT GENERATE HUMAN MONOCLONAL ANTI-CTLA-4 ANTIBODIES

The present invention also provides transgenic non-human animals, *e.g.*, a transgenic mice, which are capable of expressing human monoclonal antibodies that specifically bind to CTLA-4. High affinity human sequence antibodies are also provided. Some transgenic non-human animals, *e.g.*, the transgenic mice, have a genome comprising a human heavy chain transgene and a light chain transgene. Some transgenic non-human animals are immunized with a purified or enriched preparation of CTLA-4 antigen and/or cells expressing CTLA-4. Some transgenic non-human animals are capable of producing multiple isotypes of human monoclonal antibodies to CTLA-4 (*e.g.*, IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching. Isotype switching may occur by, *e.g.*, classical or non-classical isotype switching.

The design of a transgenic non-human animal that responds to foreign antigen stimulation with a heterologous antibody repertoire, requires that the heterologous immunoglobulin transgenes contained within the transgenic animal function correctly throughout the pathway of B-cell development. In some mice, correct function of a heterologous heavy chain transgene includes isotype switching. Accordingly, the transgenes of the invention are constructed so as to produce isotype switching and one or more of the following: (1) high level and cell-type specific expression, (2) functional gene rearrangement, (3) activation of and response to allelic exclusion, (4) expression of a

sufficient primary repertoire, (5) signal transduction, (6) somatic hypermutation, and (7) domination of the transgene antibody locus during the immune response.

Not all of the foregoing criteria need be met. For example, in transgenic animal in which the endogenous immunoglobulin loci of the transgenic animals are functionally disrupted, the transgene need not activate allelic exclusion. Further, in transgenic animals in which the transgene comprises a functionally rearranged heavy and/or light chain immunoglobulin gene, the second criteria of functional gene rearrangement is unnecessary, at least for that transgene which is already rearranged. For background on molecular immunology, *See, e.g.*, *Fundamental Immunology*, 4th edition (1998), Paul, William E., ed. Lippencott-Raven Press, N.Y.

Some transgenic non-human animals used to generate the human monoclonal antibodies of the invention contain rearranged, unrearranged or a combination of rearranged and unrearranged heterologous immunoglobulin heavy and light chain transgenes in the germline of the transgenic animal. Each of the heavy chain transgenes comprises at least one CH gene. In addition, the heavy chain transgene can contain functional isotype switch sequences, which are capable of supporting isotype switching of a heterologous transgene encoding multiple CH genes in the B-cells of the transgenic animal. Such switch sequences can be those which occur naturally in the germline immunoglobulin locus from the species that serves as the source of the transgene CH genes, or such switch sequences can be derived from those which occur in the species that is to receive the transgene construct (the transgenic animal). For example, a human transgene construct that is used to produce a transgenic mouse may produce a higher frequency of isotype switching events if it incorporates switch sequences similar to those that occur naturally in the mouse heavy chain locus, as presumably the mouse switch sequences are optimized to function with the mouse switch recombinase enzyme system, whereas the human switch sequences are not. Switch sequences can be isolated and cloned by conventional cloning methods, or can be synthesized de novo from overlapping synthetic oligonucleotides designed on the basis of published sequence information relating to immunoglobulin switch region sequences (Mills *et al..*, *Nucl. Acids Res.* 15:7305-7316 (1991); Sideras *et al..*, *Intl. Immunol.* 1:631-642 (1989).

For each of the foregoing transgenic animals, functionally rearranged heterologous heavy and light chain immunoglobulin transgenes are found in a significant fraction of the B-cells of the transgenic animal (at least 10 percent).

The transgenes used to generate the transgenic animals of the invention include a heavy chain transgene comprising DNA encoding at least one variable gene segment, one diversity gene segment, one joining gene segment and at least one constant region gene segment. The immunoglobulin light chain transgene comprises DNA encoding at least one variable gene segment, one joining gene segment and at least one constant region gene segment. The gene segments encoding the light and heavy chain gene segments are heterologous to the transgenic non-human animal in that they are derived from, or correspond to, DNA encoding immunoglobulin heavy and light chain gene segments from a species not consisting of the transgenic non-human animal. In one aspect of the invention, the transgene is constructed such that the individual gene segments are unrearranged, *i.e.*, not rearranged so as to encode a functional immunoglobulin light or heavy chain. Such unrearranged transgenes support recombination of the V, D, and J gene segments (functional rearrangement) and preferably support incorporation of all or a portion of a D region gene segment in the resultant rearranged immunoglobulin heavy chain within the transgenic non-human animal when exposed to CTLA-4 antigen.

Such transgenes typically comprise a substantial portion of the C, D, and J segments as well as a subset of the V gene segments. In such transgene constructs, the various regulatory sequences, *e.g.* promoters, enhancers, class switch regions, splice-donor and splice-acceptor sequences for RNA processing, recombination signals and the like, comprise corresponding sequences derived from the heterologous DNA. Such regulatory sequences may be incorporated into the transgene from the same or a related species of the non-human animal used in the invention. For example, human immunoglobulin gene segments may be combined in a transgene with a rodent immunoglobulin enhancer sequence for use in a transgenic mouse. Alternatively, synthetic regulatory sequences may be incorporated into the transgene, wherein such synthetic regulatory sequences are not homologous to a functional DNA sequence that is known to occur naturally in the genomes of mammals. Synthetic regulatory sequences are designed according to consensus rules, such as, for example, those specifying the permissible sequences of a splice-acceptor site or a promoter/enhancer motif. The transgene may comprise a minilocus.

Some transgenic animals used to generate human antibodies to CTLA-4 contain at least one, typically 2-10, and sometimes 25-50 or more copies of the transgene described in Example 37 of US patent 5,770,429, or the transgene described in Example 2

below (*e.g.*, HCo12), at least one copy of a light chain transgene described in Examples 38 of US patent 5,770,429, two copies of the Cmu deletion described in Example 1 below, and two copies of the Jkappa deletion described in Example 9 of US patent 5,770,429. The resultant animals are injected with antigens and used for production of human monoclonal antibodies against these antigens.

Some transgenic animals exhibit immunoglobulin production with a significant repertoire, ideally substantially similar to that of a native mouse. Thus, for example, animals in which the endogenous Ig genes have been inactivated, the total immunoglobulin levels range from about 0.1 to about 10 mg/ml of serum.

The immunoglobulins expressed by the transgenic mice typically recognize about one-half or more of highly antigenic proteins, *e.g.*, staphylococcus protein A. Typically, the immunoglobulins exhibit an association constant for preselected antigens of at least about 10^7M^{-1}, 10^8M^{-1}, 10^9M^{-1}, $10^{10}M^{-1}$, $10^{11}M^{-1}$, $10^{12}M^{-1}$, $10^{13}M^{-1}$, or greater.

The transgenic mice of the present invention can be immunized with a purified or enriched preparation of human CTLA-4 antigen (or antigenic fragment thereof) and/or cells expressing human CTLA-4 as described previously. The mice produce B cells that undergo class-switching via intratransgene switch recombination (*cis*-switching) and express immunoglobulins reactive with CTLA-4. The immunoglobulins can be human sequence antibodies, wherein the heavy and light chain polypeptides are encoded by human transgene sequences, which may include sequences derived by somatic mutation and V region recombinatorial joints, as well as germline-encoded sequences; these human sequence immunoglobulins can be referred to as being substantially identical to a polypeptide sequence encoded by a human VL or VH gene segment and a human JL or JH segment, even though other non-germline sequences may be present as a result of somatic mutation and differential V-J and V-D-J recombination joints. With respect to such human sequence antibodies, the variable regions of each chain are typically at least 80 percent encoded by human germline V, J, and, in the case of heavy chains, D, gene segments; frequently at least 85 percent of the variable regions are encoded by human germline sequences present on the transgene; often 90 or 95 percent or more of the variable region sequences are encoded by human germline sequences present on the transgene. However, since non-germline sequences are introduced by somatic mutation and VJ and VDJ joining, the human sequence antibodies frequently have some variable region sequences (and less frequently constant region sequences) which are not

encoded by human V, D, or J gene segments as found in the human transgene(s) in the germline of the mice. Typically, such non-germline sequences (or individual nucleotide positions) cluster in or near CDRs, or in regions where somatic mutations are known to cluster.

The human sequence antibodies which bind to the predetermined antigen can result from isotype switching, such that human antibodies comprising a human sequence γ chain (such as γl, γ2, γ3, or γ4) and a human sequence light chain (such as kappa or lambda) are produced. Such isotype-switched human sequence antibodies often contain one or more somatic mutation(s), typically in the variable region and often in or within about 10 residues of a CDR) as a result of affinity maturation and selection of B cells by antigen, particularly subsequent to secondary (or subsequent) antigen challenge. Some high affinity human sequence antibodies have equilibrium association constants of at least about $1 \times 10^7\ M^{-1}$, or at least about $1 \times 10^8\ M^{-1}$, or more than about $1 \times 10^9\ M^{-1}$, or $5 \times 10^9\ M^{-1}$ to $1 \times 10^{11}\ M^{-1}$ or greater.

Another aspect of the invention pertains to the B cells from such mice which can be used to generate hybridomas expressing human monoclonal antibodies which bind with high affinity (*e.g.*, having association constant of greater than $10^7 M^{-1}$) to CTLA-4. These hybridomas are used to generate a composition comprising an immunoglobulin having an association constant (Ka) of at least $10^7\ M^{-1}$ for binding CTLA-4. Such immunoglobulin contains a human sequence light chain composed of a light chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human Vk or Vλ gene segment and a human Jk or Jλ segment, and a light chain constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human Ck or Cλ gene segment. It also contains a human sequence heavy chain composed of a heavy chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human VH gene segment, optionally a D region, and a human JH segment, and a constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human CH gene segment.

The invention also provides human monoclonal antibodies and human sequence antibodies to human CTLA-4 derivatized or linked to another functional molecule, *e.g.*, another peptide or protein (*e.g.*, a cytokine, a cytotoxic agent, an immune stimulatory or inhibitory agent, a Fab' fragment, and the like, as discussed above) to

encoded by human V, D, or J gene segments as found in the human transgene(s) in the germline of the mice. Typically, such non-germline sequences (or individual nucleotide positions) cluster in or near CDRs, or in regions where somatic mutations are known to cluster.

The human sequence antibodies which bind to the predetermined antigen can result from isotype switching, such that human antibodies comprising a human sequence γ chain (such as γ1, γ2, γ3, or γ4) and a human sequence light chain (such as kappa or lambda) are produced. Such isotype-switched human sequence antibodies often contain one or more somatic mutation(s), typically in the variable region and often in or within about 10 residues of a CDR) as a result of affinity maturation and selection of B cells by antigen, particularly subsequent to secondary (or subsequent) antigen challenge. Some high affinity human sequence antibodies have equilibrium association constants of at least about 1×10^7 M^{-1}, or at least about 1×10^8 M^{-1}, or more than about 1×10^9 M^{-1}, or 5×10^9 M^{-1} to 1×10^{11} M^{-1} or greater.

Another aspect of the invention pertains to the B cells from such mice which can be used to generate hybridomas expressing human monoclonal antibodies which bind with high affinity (*e.g.*, having association constant of greater than $10^7 M^{-1}$) to CTLA-4. These hybridomas are used to generate a composition comprising an immunoglobulin having an association constant (Ka) of at least 10^7 M^{-1} for binding CTLA-4. Such immunoglobulin contains a human sequence light chain composed of a light chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human Vk or Vλ gene segment and a human Jk or Jλ segment, and a light chain constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human Ck or Cλ gene segment. It also contains a human sequence heavy chain composed of a heavy chain variable region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human VH gene segment, optionally a D region, and a human JH segment, and a constant region having a polypeptide sequence which is substantially identical to a polypeptide sequence encoded by a human CH gene segment.

The invention also provides human monoclonal antibodies and human sequence antibodies to human CTLA-4 derivatized or linked to another functional molecule, *e.g.*, another peptide or protein (*e.g.*, a cytokine, a cytotoxic agent, an immune stimulatory or inhibitory agent, a Fab' fragment, and the like, as discussed above) to

generate a bispecific or multispecific molecule which binds to multiple binding sites or target epitopes. For example, an antibody or antigen-binding portion of the invention can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic.

Accordingly, the present invention includes bispecific and multispecific composition comprising at least one human sequence antibody or antigen binding fragment with a first binding specificity for human CTLA-4 and a second binding specificity for a second target epitope. The second target epitope can be an Fc receptor, *e.g.*, human FcγRI or a human Fcγ receptor. Therefore, the invention includes bispecific and multispecific molecules capable of binding both to FcγR1, FcγR or FcεR expressing effector cells (*e.g.*, monocytes, macrophages or polymorphonuclear cells (PMNs)), and to target cells expressing human CTLA-4. These multi-specific (*e.g.*, bispecific or multispecific) molecules target human CTLA-4 expressing cells to effector cells, and trigger Fc receptor-mediated effector cell activities, such as phagocytosis of a human CTLA-4-expressing cells, antibody dependent cell-mediated cytotoxicity (ADCC), cytokine release, or generation of superoxide anion.

The bispecific and multispecific molecules of the invention can comprise a binding specificity at least one antibody, or an antibody fragment thereof, including, *e.g.*, an Fab, Fab', $F(ab')_2$, Fv, or a single chain Fv. The antibody may also be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct as described in, e.g, Ladner *et al.*. U.S. Patent No. 4,946,778. Bispecific and multispecific molecules of the invention can comprise a binding specificity for an FcγR or an FcγR present on the surface of an effector cell, and a second binding specificity for a target cell antigen, *e.g.*, human CTLA-4.

The binding specificity for an Fc receptor is provided by a monoclonal antibody, the binding of which is not blocked by human immunoglobulin G (IgG). As used herein, the term "IgG receptor" refers to any of the eight γ-chain genes located on chromosome 1. These genes encode a total of twelve transmembrane or soluble receptor isoforms which are grouped into three Fcγ receptor classes: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). For example, the Fcγ receptor can be a human high affinity FcγRI. The human FcγRI is a 72 kDa molecule, which shows high affinity for monomeric IgG (10^8 to $10^9 M^{-1}$).

The production and characterization of these preferred monoclonal antibodies are described by Fanger *et al.*. in PCT application WO 88/00052 and in U.S. Patent No. 4,954,617. These antibodies bind to an epitope of FcγRI, FcγRII or FcγRIII at a site which is distinct from the Fcγ binding site of the receptor and, thus, their binding is not blocked substantially by physiological levels of IgG. Specific anti-FcγRI antibodies useful in this invention are MAb 22, MAb 32, MAb 44, MAb 62 and MAb 197. The hybridoma producing MAb 32 is available from the American Type Culture Collection, ATCC Accession No. HB9469. Anti-FcγRI MAb 22, $F(ab')_2$ fragments of MAb 22, and can be obtained from Medarex, Inc. (Annandale, N.J.). In other embodiments, the anti-Fc γ receptor antibody is a humanized form of monoclonal antibody 22 (H22). The production and characterization of the H22 antibody is described in Graziano (1995) J. Immunol 155:4996-5002 and PCT/US93/10384. The H22 antibody producing cell line was deposited at the American Type Culture Collection on November 4, 1992 under the designation HA022CL1 and has the accession no. CRL 11177.

The binding specificity for an Fc receptor can also be provided by an antibody that binds to a human IgA receptor, *e.g.*, an Fc-alpha receptor (FcαR (CD89)). Preferably, the antibody binds to a human IgA receptor at a site that is not blocked by endogenous IgA. The term "IgA receptor" is intended to include the gene product of one α-gene (FcαRI) located on chromosome 19. This gene is known to encode several alternatively spliced transmembrane isoforms of 55 to 110 kDa. FcαRI (CD89) is constitutively expressed on monocytes/ macrophages, eosinophilic and neutrophilic granulocytes, but not on non-effector cell populations. FcαRI has medium affinity ($\approx 5 \times 10^7\ M^{-1}$) for both IgA1 and IgA2, which is increased upon exposure to cytokines such as G-CSF or GM-CSF (Morton (1996) Critical Reviews in Immunology 16:423-440). Four FcαRI-specific monoclonal antibodies, identified as A3, A59, A62 and A77, which bind FcαRI outside the IgA ligand binding domain, have been described by, e.g, Monteiro (1992) *J. Immunol.* 148:1764.

Bispecific and multispecific molecules of the invention can further comprise a binding specificity which recognizes, *e.g.*, binds to, a target cell antigen, *e.g.* human CTLA-4. The binding specificity is provided by a human sequence antibody or a human monoclonal antibody of the present invention.

An "effector cell specific antibody" as used herein refers to an antibody or functional antibody fragment that binds the Fc receptor of effector cells. Preferred

antibodies for use in the subject invention bind the Fc receptor of effector cells at a site which is not bound by endogenous immunoglobulin.

As used herein, the term "effector cell" refers to an immune cell which is involved in the effector phase of an immune response, as opposed to the cognitive and activation phases of an immune response. Exemplary immune cells include a cell of a myeloid or lymphoid origin, *e.g.*, lymphocytes (*e.g.*, B cells and T cells including cytolytic T cells (CTLs)), killer cells, natural killer cells, macrophages, monocytes, eosinophils, neutrophils, polymorphonuclear cells, granulocytes, mast cells, and basophils. Effector cells express specific Fc receptors and carry out specific immune functions. An effector cell can induce antibody-dependent cell-mediated cytotoxicity (ADCC), *e.g.*, a neutrophil capable of inducing ADCC. For example, monocytes, macrophages, neutrophils, eosinophils, and lymphocytes which express FcαR are involved in specific killing of target cells and presenting antigens to other components of the immune system, or binding to cells that present antigens. An effector cell can also phagocytose a target antigen, target cell, or microorganism.

The expression of a particular FcR on an effector cell can be regulated by humoral factors such as cytokines. For example, expression of FcγRI has been found to be up-regulated by interferon gamma (IFN-γ). This enhanced expression increases cytotoxic activity (including, *e.g.*, phagocytosis) by FcγRI-bearing cells against target cells.

"Target cell" shall mean any undesirable cell in a subject (*e.g.*, a human or animal) that can be targeted by a composition (*e.g.*, a human sequence antibody or a human monoclonal antibody of the invention, a bispecific or a multispecific molecule of the invention). The target cell can be a cell expressing or overexpressing human CTLA-4. Cells expressing human CTLA-4 can include tumor cells, *e.g.* lymphomas.

In addition to human sequence antibodies and human monoclonal antibodies of the invention, other antibodies can be also be employed in the bispecific or multispecific molecules of the invention, including, *e.g.*, murine, chimeric and humanized monoclonal antibodies.

Chimeric mouse-human monoclonal antibodies (*i.e.*, chimeric antibodies) can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine

Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted. (*See, e.g.*, Robinson *et al.*., International Patent Publication PCT/US86/02269; Akira, *et al.*, European Patent Application 184,187; Taniguchi, M.,